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THE EFFECT OF SOME HERBICIDES ON SPORULATION OF BARLEY LEAF PATHOGENS

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "The Effect of Some Herbicides on the Sporulation of Barley Leaf Pathogens", submitted by Wen-Jou Wang Kao in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Conidia are the chief infecting agents of fungal pathogens of barley leaves. An inhibition or complete suppression of sporulation would, therefore, reduce or preclude the disease potential of each pathogen accordingly.

Five hercide formulations, viz., 2,4-D ester, 2,4-D amine, MCPA ester, MCPA amine and Carbyne were tested for their effect on sporulation of three fungal pathogens of barley leaves.

Sporulation of Rhynchosporium secalis (scald) in culture was reduced or suppressed entirely by the vapor phase of the ester forms and of Carbyne, especially at concentrations of 500 ppm and above. The amine forms, having low volatility, were ineffective. The vapor phase suppressed sporulation of mycelia in leaf lesions only slightly even at the highest concentrations.

When herbicides were applied in liquid form to R. secalis in culture or on leaves, the suppression or elimination of sporulation was quite pronounced even at low concentrations.

There was no apparent change in the amount of total growth of the fungus in all herbicides except barban. Conidial production was suppressed in various degrees depending on the herbicide and the concentration used. Mycelial growth merely replaced conidial development. Suppression of sporulation by Carbyne was usually related to reduction in growth of mycelium.

Cultures of R. secalis, which had lost their ability to sporulate, did not regain this property after numerous transfers in a spore-inducing medium or after exposure to ultra-violet light. Mycelia in scald lesions, however, regained the ability to sporulate, at least partly in some instances, after dry storage for 30 or 60 days.

Sporulation in culture of Drechslera teres (net blotch) or of Bipolaris sorokiniana (spot blotch) was inhibited less than that of R. secalis. Partial inhibition or complete loss of sporulation was closely related to suppression of mycelial growth in most instances.

The sodium salt of 2,4-D (active ingredient) behaved somewhat similarly to the complete formulations when used in liquid state.

The solvent portion of Carbyne suppressed sporulation slightly more than the active ingredient of this herbicide.

Infection of excised barley leaves by D. teres or by B. sorokiniana did not occur in leaves treated with 5,000 or 10,000 ppm of the ester forms or of Carbyne. Lack of leaf infection was due to the failure of conidia to germinate or of germinating hyphae to form appressoria.

ACKNOWLEDGEMENTS

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INTRODUCTION

The success of a fungal pathogen in producing disease depends on its ability to survive adverse conditions, to sporulate abundantly, to germinate vigorously, and to establish a parasitic relationship with its plant host. An interference with any or a combination of these processes would reduce the disease potential of the pathogen accordingly.

It is well known that sporulation of many plant pathogenic fungi is sensitive to minor nutritional and environmental changes that do not appreciably affect mycelial development. If sporulation in nature is likewise a more sensitive process than growth, it would appear to be appropriate to continue and to intensify the search for specific chemical inhibitors of sporulation. The reduction of inoculum potential might constitute an easier and more efficient means of controlling some plant diseases than attempting to suppress spore germination and infection by use of protectant fungicides. It would be even more practical if the effective chemical were one that is already used for another purpose, for example, a herbicide used for weed control.

Perhaps the greatest importance of spores as disseminating and infecting agents is to be found in fungal leaf pathogens. Barley leaves are subject to attack by a number of fungi. Among these, Rhynchosporium secalis (Oud.) J.J. Davis, causing scald (Fig. 1A), and Drechslera teres (Sacc.) Shoemaker, causing net blotch (Fig. 1C)

are the most damaging leaf diseases of barley in Alberta and in most of Saskatchewan. Bipolaris sorokiniana (Sacc. in Sorok.) Shoemaker, causing spot blotch (Fig. 1B), is more prevalent in Manitoba than in the other two prairie provinces. This pathogen is, however, more important as the cause of common root-rot and kernel discoloration in the barley and wheat crops of the prairie provinces.

There is a large amount of inoculum of these three pathogens in the barley debris in barley fields where proper rotation is not practised. The inoculum usually overwinters as dormant mycelium in the straw. In spring spores are produced and initiate primary infections. During the growing season new spores are produced in the lesions of growing plants and thus furnish sufficient inoculum to develop epidemic proportions of disease under favorable environmental conditions.

In addition to seed treatment and crop rotation, the most practical approach to the control of these diseases would be through the suppression of conidial development in the primary lesions by means of chemical spray.

The time of application of herbicides for the control of weeds in cereal crops often coincides with the period during which fungal leaf pathogens are actively sporulating and initiating infections. These herbicidal compounds may produce various effects on certain higher plants because of their growth-regulating properties. Since fungi are plants, it is reasonable to assume that herbicides may affect some physiological processes involved in their growth and reproduction.

The purpose of this investigation was to determine the effects of different concentrations of five widely-used herbicides on the sporulation of R. secalis, D. teres and B. sorokiniana.

Fig. 1. Barley leaf symptoms. A. Scald, caused by Rhynchosporium secalis. B. Spot blotch, caused by Bipolaris sorokiniana. C. Net blotch, caused by Drechslera teres.

LITERATURE REVIEW

In 1955 Horsfall and Rich (12) became interested in compounds that might selectively inhibit sporulation as a possible means of plant disease control. They compared a number of compounds, including mitotic poisons, chelating agents, and others as to their relative effectiveness in reducing mycelial growth and in inhibiting production of conidia by Monilinia fructicola. Some of the mitotic poisons showed greater inhibitory activity toward sporulation than toward mycelial growth, as did also a number of the chelating agents. Several compounds, including ketones, acids, and amides actually enhanced sporulation, while among the compounds that were most active in selectively suppressing sporulation in these experiments were sodium thiocyanate, benzidine hydrochloride, diphenylthiocarbazone, and bis(4-dimethylaminophenyl)methane.

Metals may promote sporulation in some instances, but in others have the opposite effect. Cobalt, for example, induces certain yeasts to grow in a filamentous form (16). Zinc is involved in the fragmentation process in cultures of Ustilago sphaerogena (9), but the exact nature of the involvement is not understood. When zinc was added to the liquid growth medium for this fungus, a culture of single ovid cells was produced. Without zinc the cells were filamentous in form. Apparently zinc was involved somehow in cellular processes related to cell division. Spoerl (24) observed an opposite effect of zinc. The strain of U. sphaerogena used in his work produced long clumped cells when zinc was added to the basic medium. Because growth was

not markedly changed, it appeared that zinc exerted its influence specifically on the process of cell division.

Compounds that react with metals might logically be expected to affect sporulation and Rich and Horsfall (19) found that the metal chelator, dimethylglyoxime, prevented sporulation in Aspergillus niger at concentrations that do not appreciably affect growth of mycelium. They found the metal-reacting oximes and quinolines to be effective in reducing the spore production of Sclerotinia fructicola, Penicillium sp., and A. niger.

Inhibition of sporulation of A. niger by thiourea (7) and in Peronospora destructor by limesulfur (27) was considered to result from interference with essential metals (11).

In 1954 Reavill (18) grew Botrytis cinerea in an atmosphere saturated with tetrachloronitrobenzene and showed that there was a retardation of germination and colony growth and that sporulation was completely suppressed. Similar effects were shown on Phytophthora parasitica, Mucor hiemalis, and Trichoderma viride, but none of these was as sensitive as Botrytis cinerea especially as regards linear growth. Of two strains of Fusarium caeruleum, one was much more sensitive than the other, both in linear growth and sporulation.

The study of the relationship of plant-growth regulators to plant disease is not much more than twenty years old, and most of the work on this problem has been on disease resistance rather than on their effects on the pathogens (21). Many plant-growth regulators are known to influence profoundly the biochemical processes of higher

plants and are relatively innocuous to certain fungi (5, 28).

Most studies on the effect of growth-regulators on fungi are concerned with spore germination and rate of growth (1, 3, 8).

Several investigators (14, 26) dealing with the responses of a number of filamentous fungi to growth-regulating substances, chiefly indole-3-acetic acid, have shown that (a) high concentrations were inhibitory or toxic and that (b) low concentrations failed to bring about any increase in growth or any other effect.

Studies on the effect of some commonly used herbicides on filamentous fungi are also mostly restricted to their effects on rate of growth and on spore germination. Lewis and Hamner (15) reported toxic effects of 2,4-D on some fungi. In 1949 Richards (20) grew four filamentous fungi in the presence of a range of concentrations of four growth-regulating substances, among which were 2,4-dichlorophenoxy-acetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5,-T). She concluded that the effect of these substances on the different fungi were of two classes, stimulating and inhibiting. These effects were not identical on the four test organisms. As a general rule inhibition occurred within the concentration range of 10^{-2} and 10^{-3} molar; stimulation within the concentration range 10^{-3} and 10^{-4} molar. Only casual reference is made to the effect of these chemicals on sporulation and, in each instance, this effect is directly related to the amount of growth of mycelium.

In a review on auxins and fungi, Gruen (10) makes several references to the effect of some common herbicides on the growth and germination of some pathogenic fungi.

There are apparently no reports concerning an intensive study on the effect of herbicides on the sporulation of fungal leaf pathogens.

MATERIALS

Test Fungi

Monoconidial isolates of Rhynchosporium secalis, Drechslera teres, and Bipolaris sorokiniana obtained from the Edmonton area were used as test fungi.

R. secalis is known to possess the asexual stage only. The conidia, borne sessilely on cells of mycelium are hyaline, 1-septate, cylindrical to ovate, with a short oblique apical beak on most spores, and measure 12-20 by 2-4 microns (4). In culture conidia are often produced in fan-like clusters with little intervening mycelium. Primary conidia may give rise to secondary and these in turn to tertiary conidia (Fig. 2). A colony grows slowly in culture and, because of its almost exclusively conidial production, it appears yeast-like (Fig. 3A). The fungus overwinters as dormant mycelium in well-defined lesions of leaves and chaff. It sporulates abundantly under cool, moist conditions (23) (Fig. 4).

D. teres is a recent name applied by Shoemaker (22) to a fungus that has long been known as Helminthosporium teres Sacc.. Both of these names are applied to the conidial (asexual) stage of the fungus. It does, however, have an ascigerous (perfect) stage and, because the sexually-produced spores are ascospores, it belongs to the Class Ascomycetes and its Latin binomial is Pyrenophora teres (Died.) Drechsl. The ascospores apparently function only in spring.

Sporulation by means of conidia is considered in this study and therefore the binomial, D. teres, is used.

Conidia are produced on conidiophores which are somewhat stouter and darker than the mycelium. They are yellowish olivaceous, thin-walled, constricted at the septa with much rounded apical cells. The basal cell is larger with a subcylindrical shape (Fig. 5). Germ tubes develop from all cells of the conidium (6). Conidia are produced in groups up to three on the apex of the conidiophore. In culture D. teres grows quickly and forms a thick mat (Fig. 3B).

B. sorokiniana is also a name recently applied by Shoemaker (22) to the conidial stage of the fungus previously known as Helminthosporium sativum Pam., King, and Bakke. In its ascigerous stage it produces ascospores and hence belongs to the Class Ascomycetes and bears the binomial Cochliobolus sativus (Ito. and Kurib.) Drechsl.

Conidia are produced singly or in groups of 2 or 3 on the apex of dark conidiophores. The conidia are slightly to distinctly curved, thick-walled, reddish to dark olivaceous brown, 1-10 septate, widest near the middle, and the ends round off abruptly (Fig. 6). Conidia germinate from apical cells only (6). In culture B. sorokiniana forms a velvety colony with an irregular margin (Fig. 3C).

Barley Variety

Moore (C.I. 7251), a barley variety highly susceptible to the three test organisms, was used in this study as a source of scald lesions, and as a test variety wherever infection by any of the three fungi was required.

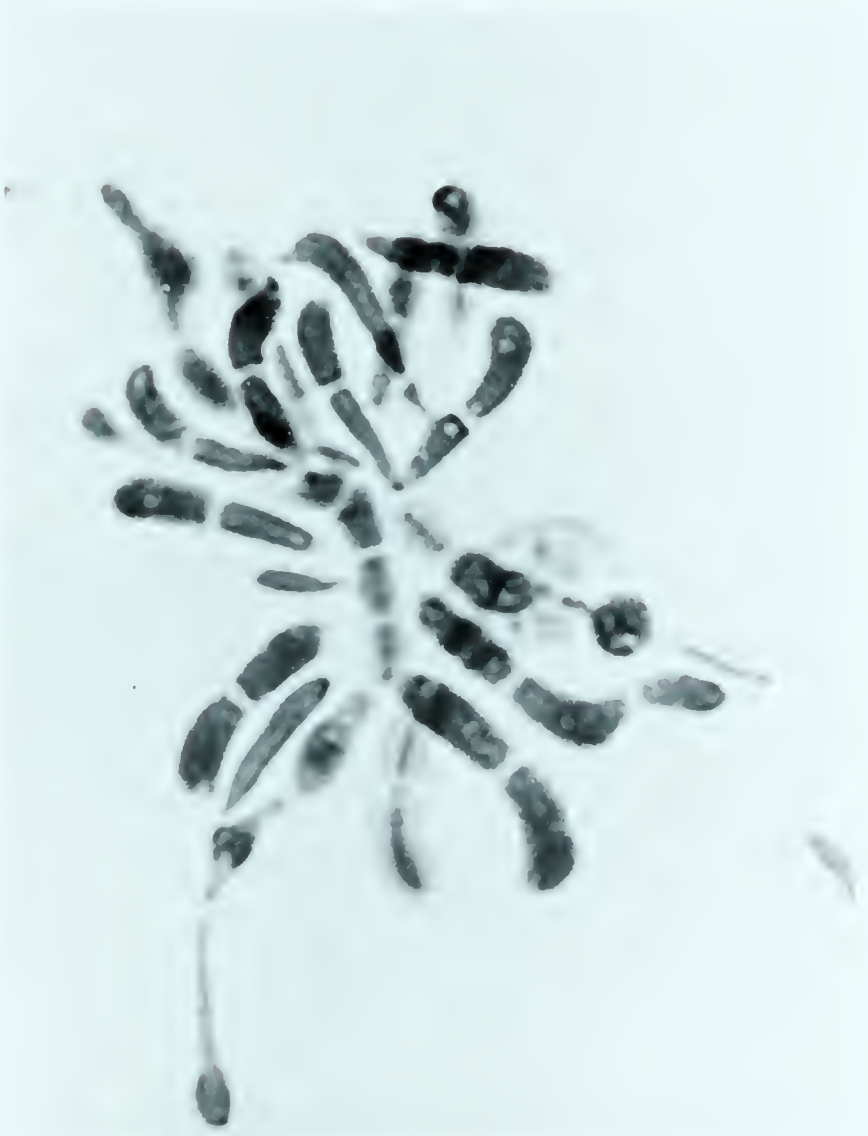

Fig. 2. Conidial colony of Rhynchosporium secalis.

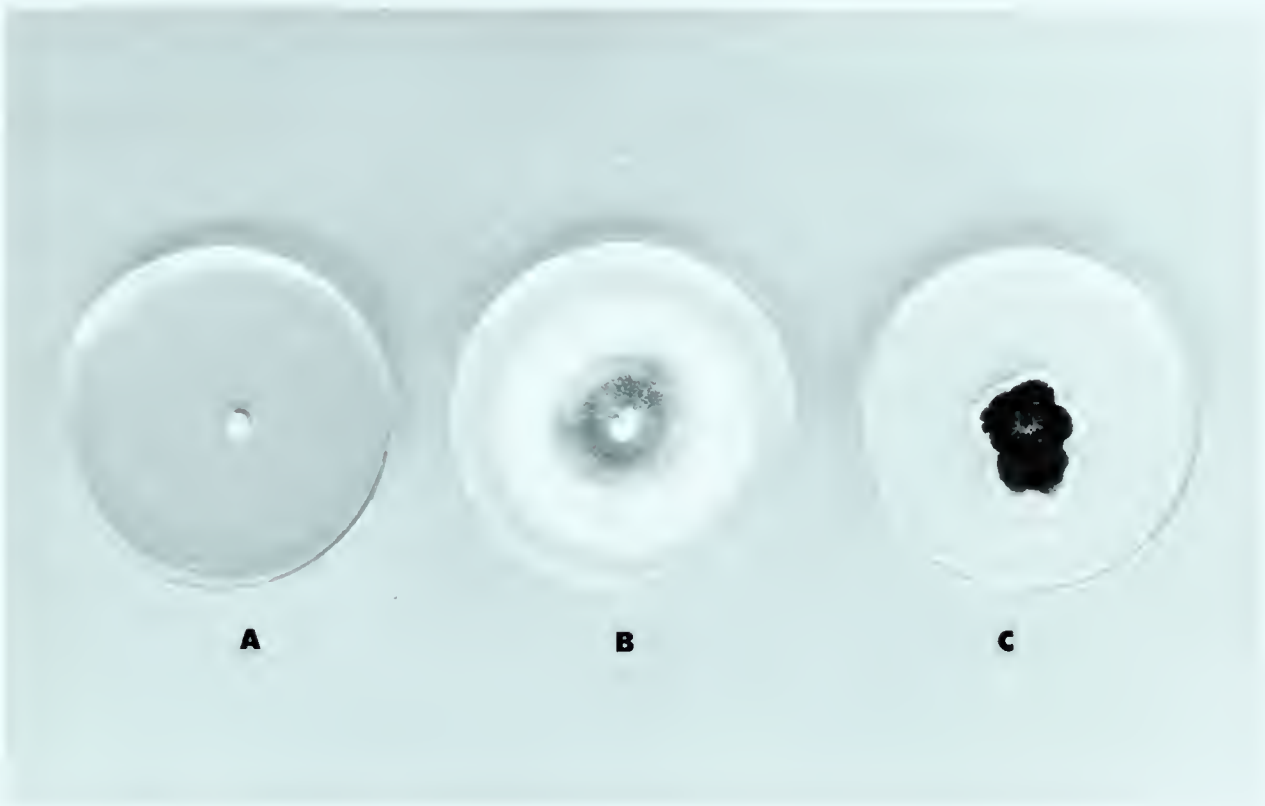

Fig. 3. Colony characteristics of: A. Rhynchosporium secalis.
B. Drechslera teres.
C. Bipolaris sorokiniana.

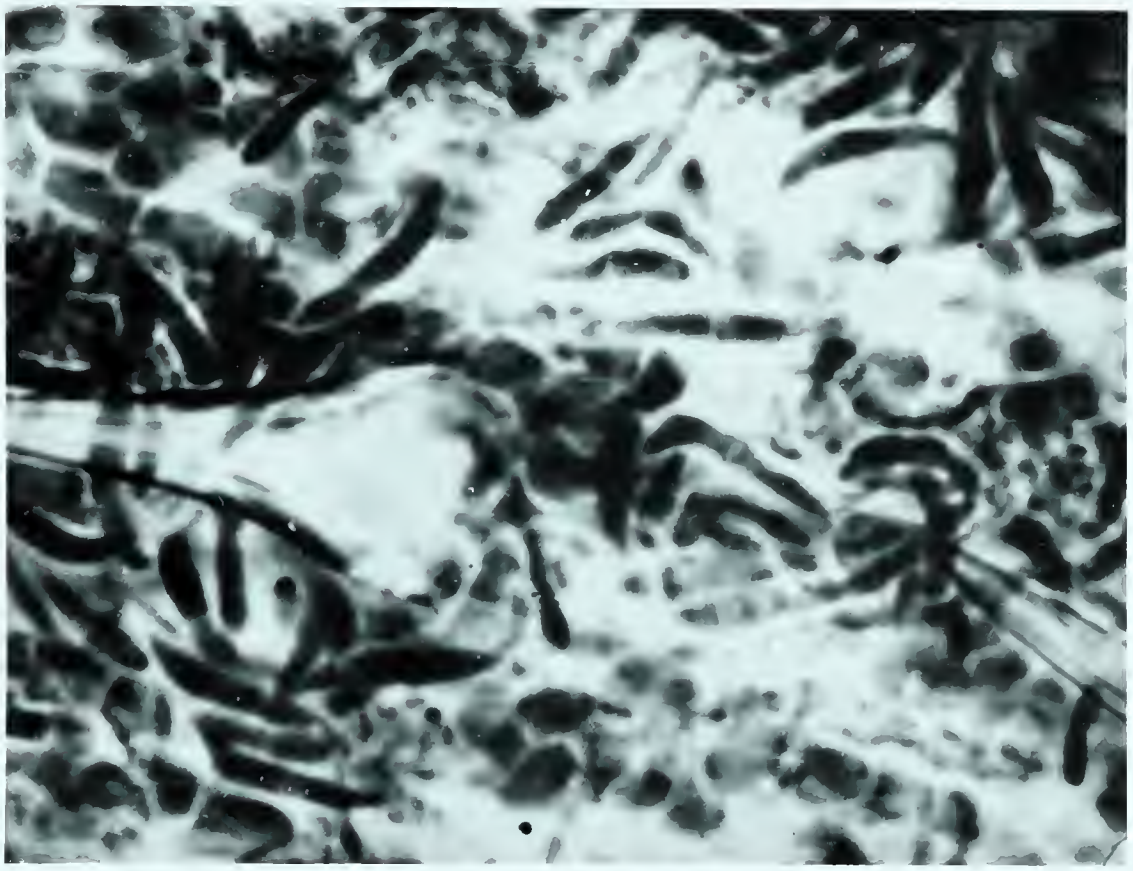

Fig. 4. Sporulation of Rhynchosporium secalis on mycelia in scald lesion.

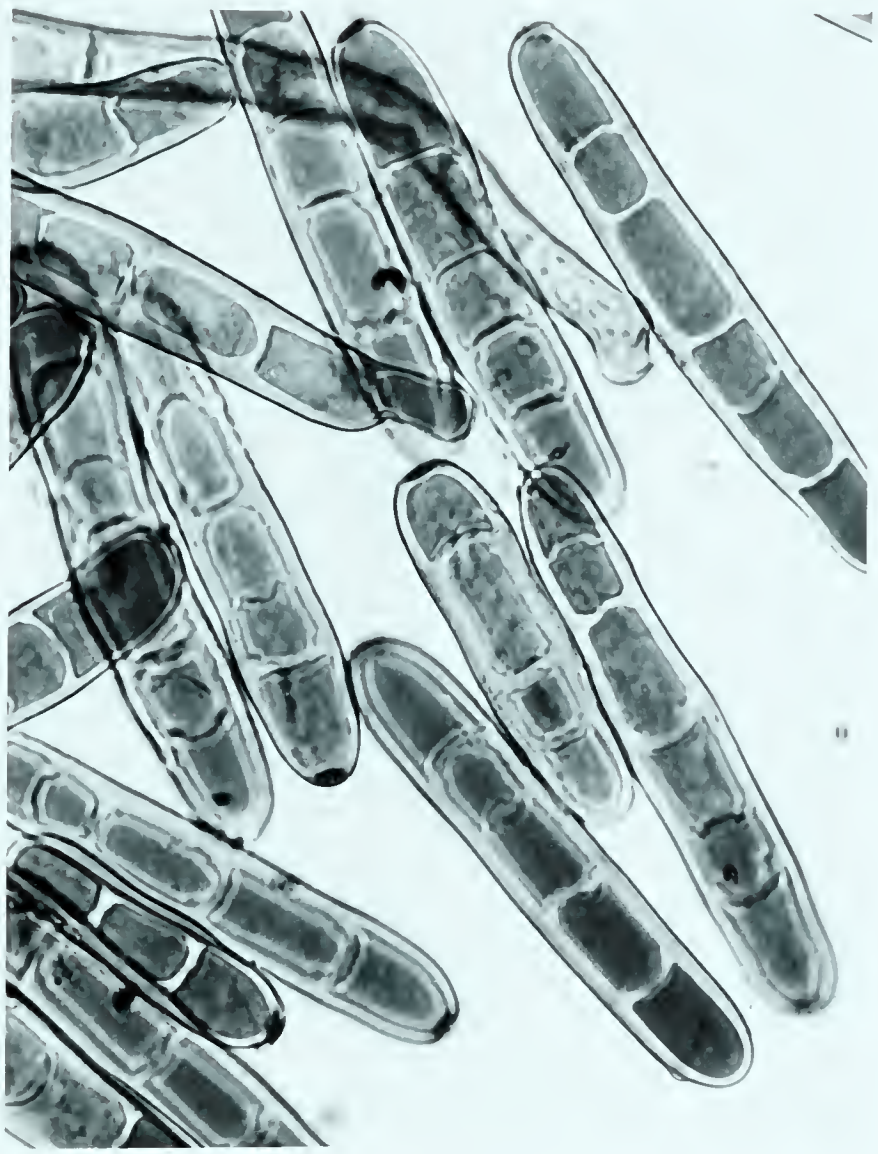

Fig. 5. Conidia of Drechslera teres.

Fig. 6. Conidia of Bipolaris sorokiniana.

Herbicides

Herbicide formulations used were : 2,4-dichlorophenoxyacetic acid, ethyl ester (2,4-D ester); 2,4-dichlorophenoxyacetic acid, alkanolamine (2,4-D amine); 2-methyl,4-chlorophenoxyacetic acid, butyl ester (MCPA ester); 2-methyl, 4-chlorophenoxyacetic acid, dimethyl amine (MCPA amine); and 4-chloro-2-butynyl N-(3-chlorophenyl) carbamate (Carbyne).

In addition to the complete herbicides, the following fractions were also used: (1) the sodium salt of 2,4-D, (2) the solvent of barban and, (3) the active ingredient of Carbyne. (Carbyne formulations were supplied by the Spencer Chemical Company, Research Center, 610 Dwight Building, Kansas City 5, Missouri).

METHODS

In all experiments the degree of sporulation was rated on a scale of 0-5, with 5 referring to maximum sporulation as obtained in the control, and 0 to no production of spores. Estimates of spore numbers were made in the following manner. To a known area of agar a known quantity of water was added and the surface gently rubbed with a wire loop to dislodge the spores. Several readings were taken in each instance.

Detailed methods used in this investigation will be given under each experiment.

RHYNCHOSPORIUM SECALIS

Vapor phase on fungus in culture

Materials and Methods

Felsen quadrant Petri dishes with 2 bisecting ridges one-quarter inch high, providing 4 shallow wells, were used. Five ml. of malt-yeast agar was poured into each of 2 opposite wells, and 2 ml. of each solution of the designated herbicide was added to each remaining well (Fig. 7). The following concentrations of herbicides were used: 5, 50, 500, 5,000, 10,000 and 50,000 ppm. Distilled water was substituted for herbicidal solutions in the controls. The agar was seeded with R. secalis and incubated at 15° C for 5 days. Each lot was replicated 4 times.

The vapor phase of each herbicide was thus responsible for any changes in sporulation because the solutions were not in contact with the nutrient agar.

TABLE 1. Effect of vapor phase of herbicides on sporulation of Rhynchosporium secalis grown on malt-yeast agar

	Degree of sporulation in concentrations (ppm)					
Herbicide	5	50	500	5,000	10,000	50,000
2,4-D amine	5	5	5	5	5	5
MCPA amine	5	5	5	5	5	5
2,4-D ester	4	3	0	0	0	0
MCPA ester	5	5	5	0	0	0
Carbyne	3	2	0	0	0	0

Results

The 5 herbicides differed considerably in their ability to inhibit sporulation (Table 1).

R. secalis sporulated abundantly in the controls and was given a rating of 5. Carbyne and 2,4-D ester suppressed sporulation completely at concentrations of 500 ppm and higher. Each one showed an increasing inhibitory effect even at concentrations beginning at 5 ppm. MCPA ester appeared to have no effect at concentrations up to 500 ppm, although it suppressed sporulation completely at higher levels.

The significant thing in this experiment is that the herbicides changed the form of fungus growth rather than the apparent total growth. The gross morphologic features of the cultures in all treatments that partly or completely inhibited sporulation, resembled the colony in controls, but in all instances the conidial production was replaced partly or completely by mycelium (Fig. 8). Carbyne, at concentrations of 5,000 ppm and above prevented growth of the fungus.

Liquid phase on fungus in culture

Methods and Materials

Fifty ml. quantities of malt-yeast medium were autoclaved in 250 ml. Erlenmeyer flasks. After cooling, quantities of each herbicide were added to give concentrations of 5, 50, 500, 5,000, 10,000 and 50,000 ppm.

Two ml. of a standard suspension of R. secalis conidia was added aseptically to each flask. The flasks were sealed to prevent

Fig. 7. Felsen quadrant Petri dish with shallow wells.

Fig. 8. Mycelial colony of Rhynchosporium secalis.

excessive loss of any volatile principle. The controls did not contain any herbicide. All lots were replicated 4 times and incubated on[a] shaker at 15° C for 10 days.

Results

All herbicidal solutions had an inhibitory effect on sporulation, but the degree of inhibition differed considerably between herbicides (Table 2). Conidia were produced abundantly in the control medium and were given a rating of 5.

2,4-D ester, MCPA ester and Carbynewere highly inhibitory throughout the entire range of concentrations, while the amine forms of 2,4-D and of MCPA had the least effects on sporulation.

In all lots, including the controls, conidia were slightly swollen, except in Carbyne where they were considerably more distorted and reduced in size.

TABLE 2. Effect of herbicides on sporulation of Rhynchosporium secalis in liquid malt-yeast medium

Herbicide	Degree of sporulation in concentrations (ppm)					
	5	50	500	5,000	10,000	50,000
2,4-D amine	3	3	3	2	1	1
MCPA amine	4	3	1	1	0	0
2,4-D ester	2	0	0	0	0	0
MCPA ester	4	1	0	0	0	0
Carbyne	1	1	1	0	0	0

Liquid phase on mycelia in lesions

Materials and Methods

Mycelium in lesions of barley leaves sporulates abundantly after 48 hours in moist conditions at cool temperatures (23).

Portions of barley leaves bearing lesions were placed in small vials on filter paper discs soaked in water containing different concentrations of each herbicide. Each lot was replicated 4 times.

Results

Sporulations of R. secalis was considerably or completely suppressed at most concentrations, but there was a significant difference in the effect between herbicides (Table 3).

Carbyne had the greatest inhibitory effect even at the low concentrations.

TABLE 3. Effect of the liquid state herbicides on sporulation of Rhynchosporium secalis in lesions of barley leaves

	Degree of sporulation in concentrations (ppm)					
Herbicide	5	50	500	5,000	10,000	50,000
2,4-D amine	5	5	5	2	2	0
MCPA amine	5	3	3	2	0	0
2,4-D ester	5	4	4	0	0	0
MCPA ester	4	2	1	0	0	0
Carbyne	3	0	0	0	0	0

Vapor phase on mycelia in lesions

Materials and Methods

A portion of barley leaf with a scald lesion was floated on 1 ml. of water in a small vial. A filter paper disc was soaked in herbicides of 5,000, 10,000, and 50,000 ppm and suspended in the vial on a string held in place by a rubber plug used to close the vial firmly (Fig. 9). In the control the filter paper disc was soaked in water.

After the material was incubated for 48 hours at 15° C the rubber plug and the paper filter disc were removed and the lesions were checked for conidia. Estimates of numbers of conidia were made by removing and crushing a given area of the lesion under a coverslip on a microscopic slide.

TABLE 4. Effect of the vapor phase of herbicides on sporulation of *Rhynchosporium secalis* in lesions of barley leaves

	Degree of sporulation at concentrations (ppm)		
Herbicide	5,000	10,000	50,000
2,4-D amine	5	5	3
MCPA amine	5	4	4
2,4-D ester	5	4	4
MCPA ester	5	4	3
Carbyne	5	4	3

Results

The vapor phase of herbicides caused a slight reduction in the amount of sporulation of *R. secalis* in lesions at the 10,000 and 50,000

ppm concentrations of herbicides (Table 4). There was a light deposit of water droplets on lesions due to some condensation of the saturated atmosphere occurring in the closed vials at 15° C.

Recovery of sporulation in artificial media

A. Altering nutrition

Materials and Methods

The mycelial cultures of R. secalis in which sporulation was completely suppressed by the vapor phase of the herbicides and in which mycelium grew well (Table 1) were used in this experiment.

These culture were transferred to tubes containing:

1) Malt yeast agar medium (on which R. secalis normally sporulates abundantly).

2) Malt yeast agar medium supplemented by each of the following amino acids at a rate of 0.20 grams nitrogen per litre:

a) glycine

b) L-valine

c) L-tryptophan

d) L-glutamine

e) L-serine

f) L-arginine

g) L-histidine monohydrochloride

h) L-isoleucine

i) DL-aspartic acid

j) L-lysine hydrochloride

k) L-alanine

Fig. 9. Vial and filter paper disc used to test effect of vapor phase of herbicides on sporulation of Rhynchosporium secalis in leaf lesions.

l) L-methionine

m) L-phenylalanine

n) L-leucine

o) L-tyrosine

p) L-proline

q) gamma amino butyric acid

r) D-glutamic acid

The pH of the medium was adjusted to near neutrality wherever necessary with HCl and KOH.

The cultures were subsequently retransferred to fresh medium 15 times at intervals of 10 days.

Results

The fungus failed to regain the ability to sporulate in all instances, and the mycelium continued to grow well.

B. Ultra-violet light

Materials and Methods

The mycelial cultures of R. secalis which had lost their ability to sporulate after treatment with the vapor phase of the herbicides (Table 1) were used for exposure to ultra-violet light rays. Ultra-violet light has been shown by several investigators (2, 13, 25) to induce sporulation in some fungi.

Malt-yeast agar in Petri plates was inoculated with a suspension of the mycelial form of the fungus and incubated for 7 days at 15° C.

The covers of the plates were removed and the colonies exposed to ultra-violet light rays* from a distance of 22 inches for 4 and 5 minutes respectively.

Results

There was a considerable reduction in the number of colonies of R. secalis exposed to ultra-violet light as compared to the controls. However, the colonies remained entirely mycelial.

Recovery of sporulation in lesions

Materials and Methods

All treatments with herbicides at concentrations which suppressed sporulation completely (Table 3) were repeated.

After 48 hours on filter paper discs soaked with appropriate concentrations of the herbicides, the lesioned areas were washed thoroughly with distilled water. These leaf portions were dried at room temperature, and stored for 30 or 60 days. Following each of these periods of time the lesions were placed for 48 hours on filter paper discs soaked in distilled water, and the relative numbers of spores were estimated.

Results

Control lesions produced conidia abundantly. The ability to sporulate was regained only partly in lesions which had been treated previously with the following concentrations of herbicides: 2,4-D ester and MCPA ester (5,000 ppm), almost complete restoration after 30 or 60

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* General Electric Germicidal ultra-violet light, G15T8, 15 watt, 16.5" tube.

days; MCPA amine (10,000 ppm), only slight restoration after 60 days, and Carbyne (50 ppm), slight restoration after 30 days. Conidial production was not restored in other instances.

Discussion

The effect of herbicides on sporulation of *Rhynchosporium secalis* was studied under four sets of conditions. These involved the effect of (i) the vapor phase on sporulation in agar culture, (ii) the vapor phase on sporulation in barley leaf lesions, (iii) herbicides in liquid culture, and (iv) herbicides applied directly to mycelia in barley lesions. A general trend of an increasing suppression of sporulation with an increased concentration of herbicides was evident in all instances except in the effect of the vapor phase on sporulation in leaf lesions. The vapor phase of herbicides suppressed sporulation only slightly and only at the two highest concentrations used.

Carbyne and the ester forms of 2,4-D and MCPA inhibited sporulation at the lowest concentrations; they also suppressed it over the widest range of dilutions employed. The effect of Carbyne however, differed from the other four herbicides because it also suppressed growth of mycelium. The degree of spore production was related to the reduction in total growth. In this respect the effect of Carbyne is similar to the findings of Richards (20) who worked with four filamentous fungi.

The ester forms of 2,4-D and MCPA were more effective in suppressing sporulation than their amine forms when only the vapor phase was involved. The ester forms are more highly volatile and they

would, therefore, be expected to be more effective under those conditions.

The amine forms showed strong inhibition of sporulation when applied directly to the fungus. The application of a herbicide directly to the fungus, whether in artificial culture or to mycelia occurring naturally on scald lesions, resulted in similar inhibitory effects on sporulation.

R. secalis differs from most filamentous fungi. It produces conidia almost exclusively under conditions used in controls in this study. Fan-like clusters of conidia arise from other conidia and, in this manner, chains of clusters with little intervening mycelium are produced. In most herbicides (except Carbyne) where sporulation was inhibited, the gross morphological features of the colony appeared similar to the controls. Conidia, however, were replaced partly or entirely with hyphae.

Three methods were employed in an attempt to induce sporulation in all cultures which had lost it completely on malt-yeast agar. Two of these attempts were based on altering the nutrient medium and one on the use of ultra-violet light.

Transferring the non-sporulating culture many times in fresh malt-yeast agar was designed to allow the fungus to eliminate whatever suppressing entity was incorporated into the mycelium as a result of exposure to the herbicide. Normal R. secalis sporulates abundantly on this medium.

The method involving the use of amino acids as supplements to malt-yeast agar was based on the assumption that the herbicides

may have acted as mutagenic agents. These agents can cause biochemical mutants and the missing nutrilite could have been an amino acid. Ultraviolet light was used because it has also been shown to induce sporulation in various fungi (2, 13, 25).

The three methods failed to restore sporulation in all instances.

The mechanism inhibiting sporulation of mycelium in leaf lesions must have been different from the one controlling sporulation in artificial culture. Storage of lesions for a month or two following loss of sporulation was sufficient to restore it at least partly in a few cases. Conidial production, however, was not regained in most cases.

The findings in this report may have important practical applications. Conidia of R. secalis are of prime importance in the initiation of infections. In the field, primary and secondary infections are caused by conidia produced on lesions of barley leaves. The ability of some herbicides to suppress sporulation in these lesions, even when used at or lower than practical field rates (approximately 10,000 ppm), could be an important factor in reducing the incidence of barley scald. The inability to restore sporulation after prolonged periods following treatment contributes further to the practical value of some herbicides apart from their weed-killing properties.

DRECHSLERA TERES

In Sachs medium

Materials and Methods

D. teres does not sporulate well when submerged in liquid. It is, therefore, impossible to use liquid shake cultures to determine the effect of herbicides on sporulation. D. teres also tends to lose its ability to sporulate when grown on a medium high in carbon content.

On Sachs agar medium this fungus produces a sparse growth of mycelium but sporulates abundantly. Sachs medium is made up as follows:

Distilled water	1000 ml
$CaCO_3$	4 gms.
$Ca(NO_3)_2.4H_2O$	1 gm.
K_2HPO_4	0.25 gm.
$MgSO_4.7H_2O$	0.25 gm.
$FeCl_3$	0.1 gm.
Agar	16 gms.

The agar medium was cooled to approximately 42° C before herbicides were added to make up concentrations of 5, 50, 500, 5,000, 10,000 and 50,000 ppm.

A scraping of conidia from a 7- to 9-day old D. teres culture in test tubes on Sachs medium was used as inoculum. The seeded medium in Petri plates was incubated at 20° C. Estimates of the relative numbers of conidia produced were made after 3 weeks.

TABLE 5. Effect of herbicides on sporulation of Drechslera teres on Sachs agar medium

Herbicide	Degree of sporulation in concentrations (ppm)						
	5	50	500	1,000	5,000	10,000	50,000
2,4-D amine	5	5	4	1	1	1	0*
MCPA amine	5	5	5	5	4	4	0*
2,4-D ester	5	5	5	4	0*	0	0*
MCPA ester	5	5	5	4	0*	0*	0*
Carbyne	5	5	4	4	1*	0*	0*

* Mycelial growth strongly reduced.

Results

Sporulation was good in all herbicides (except 2,4-D amine) at concentrations up to 1,000 ppm (Table 5). At 50,000 ppm there was practically no growth of mycelium and hence no spores. At 5,000 and 10,000 ppm there were two different effects on the fungus. Mycelial growth was strongly suppressed in 2,4-D ester, MCPA ester and Carbyne. With the amine forms of 2,4-D and MCPA, there was a good growth of mycelium but good sporulation occurred only with MCPA. With 2,4-D amine the amount of sporulation dropped sharply at 1,000, 5,000 and 10,000 ppm even though growth of mycelium was good.

Besides the effect of some herbicides on sporulation of D. teres there were some alterations in spore characteristics. With the ester forms of 2,4-D and MCPA at 500 and 1,000 ppm the conidia were reduced in size so that the majority of them contained three cells (Fig. 10) rather than the normal five or six.

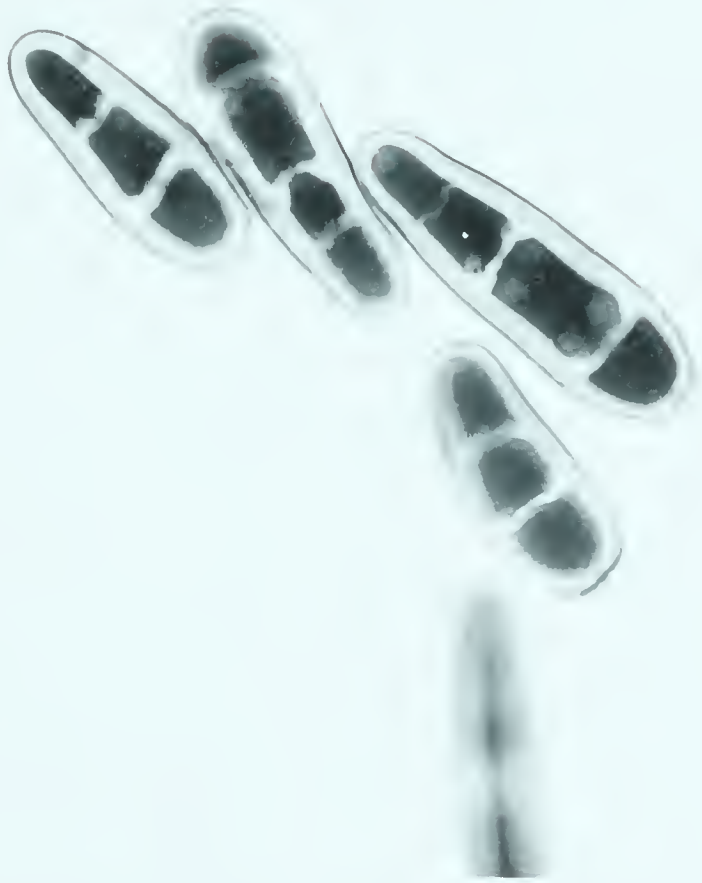

Fig. 10. Conidia of Drechslera teres reduced in size by treatment with some concentrations of the ester forms of 2,4-D or MCPA.

Vapor phase

Materials and Methods

Felsen quadrant Petri dishes with two bisecting ridges, providing four shallow wells were used. Five ml. of Sachs agar were poured into each of 2 opposite wells, and 2 ml. of each solution of the designated herbicide were added to each remaining well. The following concentrations of herbicides were used: 5, 50, 500, 1,000, 5,000, 10,000, and 50,000 ppm. Distilled water was used in the controls. The agar was seeded with conidia from an 8-day old culture of D. teres on Sachs medium. Estimates of the relative numbers of conidia were made after 2 weeks on 4 replicates of each concentration.

TABLE 6. Effect of vapor phase of herbicides on sporulation of Drechslera teres on Sachs agar medium

	Degree of sporulation in concentrations (ppm)						
Herbicide	5	50	500	1,000	5,000	10,000	50,000
2,4-D amine	5	4	4	4	3	3	1
MCPA amine	5	4	4	4	3	3	1
2,4-D ester	5	4	4	4	3	2	1
MCPA ester	5	4	4	4	2	1	0
Carbyne	5	4	3	3	1	1	0

Results

The vapor phase of most herbicides showed little effect on sporulation or growth of D. teres at concentrations up to 1,000 ppm (Table 6). Above this concentration the reduction in sporulation

was closely associated with the reduction in growth of mycelium. Barban affected mycelial growth most significantly and at the lowest concentration.

Sodium salt of 2,4-D

Materials and Methods

The procedure was the same as that described under the experiment titled: "Effect of herbicides on Sachs agar medium", with the difference that only the sodium salt of 2,4-D was used as the herbicide.

Results

The estimates on the relative numbers of conidia are given in brackets following the concentration in ppm of the sodium salt of 2,4-D: 5 (5), 50 (5), 500 (4), 1,000 (4), 5,000 (4), 10,000 (1), 50,000 (0).

Growth of mycelium of D. teres was reduced significantly at the two highest concentrations and conidial production was related to some extent to the reduction in total growth.

Active ingredient and solvent of barban

Materials and Methods

The active ingredient, 4-chloro-2-butynyl-N-(3-chlorophenyl) carbamate "Barban", 98.8 ± 0.4 per cent pure and the "Carbyne" formulation minus the active ingredient were used. Otherwise the procedure was similar to that described under the experiment dealing with the "Effect of herbicides on Sachs agar medium".

TABLE 7. Effects of active ingredient and of solvent of barban on sporulation of Drechslera teres on Sachs agar medium

	Degree of sporulation in concentrations (ppm)						
Chemical	5	50	500	1,000	5,000	10,000	50,000
Active ingredient	5	5	5	3	3	0	0
Solvent	5	5	3	0	0	0	0
Carbyne*	5	5	4	4	1	0	0

* Carbyne is included for comparison.

Results

The active ingredient affected sporulation of D. teres somewhat similarly to the Carbyne , but the solvent portion appeared to be slightly more effective (Table 7).

Drastic reductions in sporulation were associated with reductions in total growth.

BIPOLARIS SOROKINIANA

Materials and Methods

The procedure is similar to that described under the same experiments concerning D. teres. The only difference being that the test fungus is B. sorokiniani. Materials and methods, therefore, are not included under each experiment.

Results in Sachs medium

TABLE 8. Effect of herbicides on sporulation of Bipolaris sorokiniana in Sachs agar medium

Herbicide	Degree of sporulation in concentrations (ppm)						
	5	50	500	1,000	5,000	10,000	50,000
2,4-D amine	5	5	5	4	4	0*	0*
MCPA amine	5	5	5	5	1*	1*	1*
2,4-D ester	5	5	5	5	4	4	0*
MCPA ester	5	5	5	5	1	1*	1*
Carbyne	5	3	3	3	3	0*	0*

* Mycelial growth strongly reduced.

The reductions in amount of sporulation were closely related to the reductions in amount of mycelial growth at the two highest concentrations of herbicides (Table 8). In concentrations of herbicides below 10,000 ppm, there was little reduction in sporulation except in the 5,000 ppm of both forms of MCPA. Carbyne caused reduction in growth even at the low concentrations.

In addition to their effects on the amount of sporulation all the herbicides altered some spore characteristics at all concentrations above 5 ppm. The chief alteration consisted of a reduction in the amount of protoplasm, the elimination of septa, and germination from middle cells (Fig. 11).

Results in vapor phase

Sporulation of B. sorokiniana was reduced by most herbicides at concentrations of 500 ppm and above (Table 9). However, all these reductions were closely related to reductions in amount of mycelium.

Some alterations in conidial characteristics involving a reduction in size of protoplasm and loss of septations were observed. The changes were, however, not as drastic and common as in those described in Table 8.

TABLE 9. Effect of vapor phase of herbicides on sporulation of Bipolaris sorokiniana on Sachs agar medium

	Degree of sporulation in concentrations (ppm)						
Herbicide	5	50	500	1,000	5,000	10,000	50,000
2,4-D amine	5	5	5	4	3	2	1
MCPA amine	5	5	4	3	2	2	2
2,4-D ester	5	5	3	2	2	2	1
MCPA ester	5	5	3	3	2	2	0
Carbyne	5	5	3	3	2	2	0

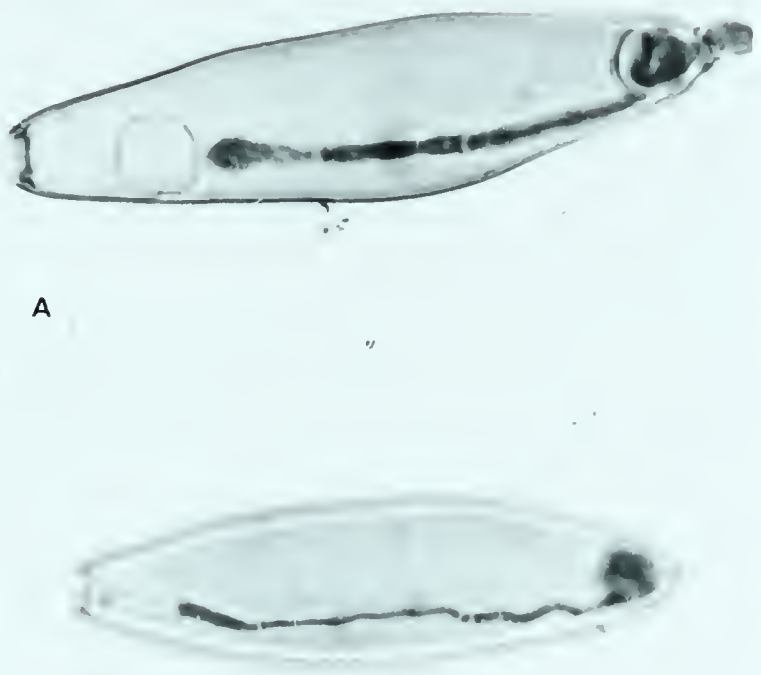

Fig. 11. Abnormalities in spore characteristics of Bipolaris sorokiniana caused by herbicides. A. Reduction in amount of protoplasm. B. Loss of septa. C. Germination from middle cells.

Sodium salt of 2,4-D

Results

Estimates on the relative numbers of conidia are given in brackets following the concentrations in ppm of this chemical: 5 (5), 50 (5), 500 (5), 1,000 (5), 5,000 (5), 10,000 (1), 50,000 (0).

The amount of mycelial growth was reduced considerably at 10,000 ppm and drastically at 50,000 ppm. Abnormalities in conidia including loss of protoplasm and septations occurred in various amounts in all concentrations of the active ingredient.

Active ingredient and solvent of Carbyne

Results

TABLE 10. Effects of active ingredient and of solvent of Carbyne on sporulation of Bipolaris sorokiniana on Sachs agar medium

	Degree of sporulation in concentration (ppm)						
Chemical	5	50	500	1,000	5,000	10,000	50,000
Active ingredient	5	5	4	4	3	0	0
Solvent	5	5	4	0	0	0	0
*Carbyne	5	3	3	3	3	0	0

* Carbyne included for comparison.

The solvent was slightly more effective in suppressing sporulation and growth at lower concentrations than the active ingredient of Carbyne (Table 10). There was practically no growth of mycelium when treated with 10,000 or 50,000 ppm of either chemical.

LEAF INFECTION BY DRECHSLERA TERES AND BY BIPOLARIS SOROKINIANA

Materials and Methods

Excised barley leaves were used to determine the effect of herbicides on infection by conidia of D. teres and of B. sorokiniana. The excised portions of leaves were maintained in a green turgid condition in Petri dishes on water containing 50 ppm of benzimidazole and 10 ppm aureomycin (17). Leaves remained apparently normal for approximately a week under these conditions.

Inoculum of D. teres and B. sorokiniana was grown on Sachs agar medium.

The leaf portions were dipped for 2 minutes in solution containing 5000 or 10,000 ppm of the herbicide. These leaves were then inoculated with a suspension of conidia from an 8-day culture. A camel's hair brush was used to apply the inoculum. The controls were dipped in tap water instead of the herbicide. Four leaves were floated on the benzimidazole-aureomycin solution in Petri dishes and incubated for 2 days at 20° C.

After the incubation period the leaves were cleared and the reason for non-infection studied.

Results

After incubation for 2 days the leaves in the controls developed distinct brown spots indicating the points at which infection and fungal development occurred. Similar infections occurred in leaves treated with 5,000 and 10,000 ppm of 2,4-D amine or MCPA amine. There were no infections on leaves treated with 5,000 or 10,000 ppm of 2,4-D ester or MCPA ester or Carbyne.

A lack of infection was due to the failure of most conidia of D. teres or of B. sorokiniana to germinate.

The small proportion of conidia that germinated failed to form appressoria and hence infection was precluded.

Discussion

The effects of herbicides on the sporulation of Drechslera teres and of Bipolaris sorokiniana are sufficiently similar that they may be treated together in the discussion.

Sachs agar medium was used because it not only induces abundant sporulation even after many transfers but it also produces conidia which closely resemble those produced under natural conditions. On PSA medium (potato sucrose agar) both fungi tend to lose their ability to sporulate after a few transfers and, in addition, the conidia are usually abnormal in size and shape. Thus PSA medium was unsuitable for this study.

The one serious disadvantage of Sachs agar medium is that it induces only a sparse amount of mycelial growth. This is a disadvantage in this study because in many instances the herbicides at their higher concentrations reduced the amount of mycelial growth. A reduction in sporulation was related to a reduction in total growth. It was difficult to estimate the degree of reduction in vegetative growth because it was sparse even in the controls.

In Sachs agar medium mycelial growth of B. sorokiniana was inhibited in various degrees only at the two highest concentrations of all herbicides, except in Carbyne. Carbyne suppressed mycelial growth

even at low concentrations. The effect on D. teres differed only in the amine forms of 2,4-D and MCPA where mycelial growth was good in all concentrations.

Some of the alterations in conidial characteristics are interesting because they represent a change in the physiology of the spore. One of the normal characteristics of B. sorokiniana is that it germinates only from the end cells. This characteristic is so constant that it is used as feature to distinguish this fungus from some species of Drechslera which germinate from all cells. The genus Bipolaris, in fact, refers to germination from the two poles only. In some herbicides germination from all cells is fairly common.

All herbicides consist of several components, one of which is the active ingredient and the other may be referred to broadly as a solvent. The effects on sporulation of D. teres and B. sorokiniana of the active ingredient of 2,4-D, i.e., the sodium salt of 2,4-dichlorophenoxyacetic acid, and the active ingredient of Carbyne and of its solvent were determined.

The sodium salt of 2,4-D was somewhat similar to the complete herbicides in that sporulation was significantly reduced at the higher concentrations and that this reduction was related to the reduction in growth of mycelium.

The results obtained with the components of Carbyne were also fairly close to the effect of Carbyne. It was significant, however, that the solvent was more effective than the active ingredient in reducing sporulation and growth of both fungi at 1,000 and 5,000 ppm

concentrations.

The effect of herbicides on the infection of barley leaves by D. teres and B. sorokiniana may be interpreted as their effect on germination of conidia. Only two concentrations, 5,000 and 10,000 ppm were used because the practical rate of field application is within that range.

Germination was inhibited and thus infection was prevented in leaves treated with 5,000 or 10,000 ppm of esters of 2,4-D or MCPA, or with Carbyne. These were generally the herbicides that were most effective in reducing sporulation. They also had the effect of reducing growth of mycelium at the higher concentrations. It may be assumed that perhaps the physiology of germination of conidia is somewhat similar to the physiology of growth of mycelium.

GENERAL DISCUSSION AND CONCLUSIONS

The physiology involved in sporulation of R. secalis appears to differ from that involved in D. teres or B. sorokiniana. All herbicides, except Carbyne suppressed sporulation of R. secalis partly or completely without apparently changing the amount of mycelial growth. This effect on sporulation was exerted at considerably lower levels of herbicides than was required for D. teres or B. sorokiniana. R. secalis may be considered to have a more sensitive mechanism inducing sporulation.

The effect of most herbicides on sporulation of D. teres and B. sorokiniana is confused with the effect on growth. The picture here is not as clear as in R. secalis insofar as the separation of sporulation from growth is concerned. This problem may be better resolved by using a nutrient agar medium on which these fungi consistently produce an abundance of mycelium and an abundance of natural-looking conidia.

From the practical point of view the suppression of sporulation or of germination is equally effective in reducing or preventing leaf infection by any of the three pathogens. Besides the practical applications that the suppression of sporulation by herbicides may have, the observations on herbicides recorded in this investigation may add to the list of agents which may be used as tools in studying the physiology of sporulation in fungi.

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